

(10)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number:

0 171 148 B1

(12)

EUROPEAN PATENT SPECIFICATION

(13) Date of publication of patent specification: 17.04.91

(51) Int. Cl.⁵ **G01N 33/543, G01N 21/77**

(21) Application number: 85304169.7

(22) Date of filing: 12.06.85

Divisional application 90202486.8 filed on
12/06/85.

(40) Devices for use in chemical test procedures.

(20) Priority: 13.06.84 GB 8415018
13.06.84 GB 8415019

(24) Date of publication of application:
12.02.86 Bulletin 86/07

(43) Publication of the grant of the patent:
17.04.91 Bulletin 91/16

(64) Designated Contracting States:
AT BE CH DE FR GB IT LI NL SE

(68) References cited:

EP-A- 0 010 456	EP-A- 0 034 049
EP-A- 0 103 426	EP-A- 0 170 375
EP-A- 0 170 376	FR-A- 2 325 920
GB-A- 2 090 659	US-A- 4 050 895
US-A- 4 327 073	

ANALYTICAL CHEMISTRY, vol. 54, no. 9, August 1982, pages 1071A-1080A, American Chemical Society, US; H. CHABAY: "Optical waveguides"

(73) Proprietor: ARES-SERONO RESEARCH & DEVELOPMENT LIMITED PARTNERSHIP
Exchange Place 37th Floor
Boston Massachusetts 02109(US)

(72) Inventor: Shanks, Ian Alexander
56 The Bury
Pavenham Bedford MK43 7TY(GB)
Inventor: Smith, Alan Martin
23 The Marsh
Carlton Bedford MK43 7JU(GB)

(74) Representative: Woodman, Derek et al
Frank B. Dehn & Co. European Patent Attorneys
Imperial House 15-19 Kingsway
London WC2B 6UZ(GB)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

Rank Xerox (UK) Business Services

EP 0 171 148 B1

Description

This invention relates to a process of manufacturing devices for use in chemical (especially biochemical or clinical) test procedures.

- 5 The devices are, in certain embodiments, intended for use in specific binding assay procedures, among which an important group is constituted by immunoassay procedures. Examples of such immuno assays, especially enzyme-linked immunoassays, are cited in Specifications Nos. EP 0 042 755, GB 2 074 727, GB 2 088 041, and GB 1 548 741.

- 10 Previously, immunoassay procedures have often been carried out using so-called microtitre wells, conventionally of about 0.5ml working capacity, amongst a variety of other liquid containers for the assay reaction liquids. Other devices and arrangements for handling immunoassay materials are described in Specifications Nos. EP 0 31 993, GB 1 571 872, GB 1 584 129 and GB 1 414 479, for example.

In particular, the prior art contains numerous disclosures of analytical devices for handling and metering small volumes of test samples.

- 15 GB 2 090 659 (Instrumentation Laboratory, Inc.) describes test strips constructed with a self-filling metering channel and a tip or inlet on which a sample of more than about 10 microlitres of for example whole blood can be placed, so that (for example) 10 microlitres is taken up by capillary action to react with a reagent carried on a fibrous pad above a filter layer beneath a transparent window. The result can be viewed by the unaided eye, e.g. as a colour reaction.

- 20 GB 2 038 075 (H E Mennier), GB 1 104 774 (J P Gallagher), EP 0 057 110, 0 034 049, 0 010 456 (Kodak), all describe some other aspect of the uses of capillary channel or chamber dimensions for handling biological or test fluids.

- GB 1 530 997 (Monsanto) describes the use of coated optical fibres which can be used in tests that change the light transmitting capabilities of the waveguides via reactions of the coatings, e.g. antigen-antibody reactions. WO 81/00912 (Buckles) also describes fibre-optic devices in which the fibre surface or surroundings modify the light transmission through the core.

USP 3 939 350 describes optical measurement of fluorescent material bound to the surface of a solid transparent prism by a method involving a single total internal reflection and interaction of the evanescent wave at the surface of the prism with the bound material.

- 30 EP 0 075 353 (Battelle) makes specific reference to the exponentially-decaying (evanescent) external radiation due to light which is propagated longitudinally in a fibre, and its interaction with coatings, and this principle is also taken up in the immunoassay test devices of EP 0 103 428 (Block) in which light of fluorescence excitation as well as emission wavelengths is propagated within an antigen - or antibody - coated optical fibre or plate contacting a capillary-dimensional sample liquid volume bounded by a tube or 35 another plate and containing a fluorescent-tagged binding partner of the material coated on the fibre or plate.

According to the invention to be described here, capillary fill cell devices can be conveniently manufactured, which facilitate in particular specific binding assays using very small liquid samples.

- 40 According to the present invention we provide a process of manufacturing specifically-reactive sample-collecting and testing devices, comprising the steps of (a) forming an immobilised specifically-reactive coating on the surface of a transparent sheet material which is capable of acting as a light transmissive waveguide and which is to provide a part of a multiplicity of the devices, (b) attaching to the said sheet material an additional structure which together with said coated sheet material provides for each device of 45 the multiplicity of devices a cavity of capillary dimension for collecting and retaining by capillarity a volume of sample liquid in contact with the specifically-reactive coating and (c) separating the assembled laminate into portions each providing one or a plurality of the sample-collecting and testing devices, such that the transparent sheet material of each device has at least one edge transverse to the plane of the sheet which is substantially optically smooth. In this process the specifically-reactive coating can be confluent or 50 continuous or divided into a pattern, e.g. of discrete portions, e.g. as a 2-dimensional array of patches, where such patches are formed they can be made for example either by first forming a continuous coating and then removing portions of it to leave the desired pattern, e.g. the array of discrete portions, or as a (for example screen-printed or flexographically-printed) array of patches.

- The devices made according to the invention are thus specifically-reactive sample-collecting and testing devices possessing a cavity or cavities each having a dimension small enough to enable sample liquid to 55 be drawn into the cavity by capillary action, wherein a surface of the cavity carries an immobilised reagent appropriate to the test to be carried out in the device, and wherein said surface is a surface of a transparent solid plate to act as a light-transmissive waveguide and forming a wall of the cavity, said plate having an edge which is substantially optically smooth and transverse, e.g. at some transverse angle but most

preferably perpendicular, to the plane of the plate.

The devices enable convenient sample collection and carrying out optical analysis in situ of the products of reaction of the sample with reagent(s) contained in the devices. The waveguide plate can be transparent to infrared, visible, and/or ultraviolet light, and one method of using the devices is to arrange for

5 a fluorescent material to become bound to the immobilised reagent to a variable extent depending on the assay and the sample material of interest, and then to carry out optical measurement of the resulting bound fluorescent material.

The immobilised reagent in the device can for example be an immobilised antigen or antibody which is capable of binding a fluorescent or luminescent or coloured component of an assay mixture. But it is understood that any immobilised material can be used that specifically interacts with another component of

10 a test reaction material in a way that gives optically measurable results, depending only on the kind of test in question.

The surface of the cavity opposite to the reagent-bearing waveguide surface can, as in examples illustrated below, be formed of a second similar transparent plate. However, this is not necessary, and for

15 some forms of test it may be suitable to use a light-absorbing or opaque or reflective opposite wall to the capillary cavity.

In some useful examples of the devices made according to the invention, a selective barrier such as a filter or dialysis membrane can be fitted to ensure that the takeup of sample liquid into the capillary-

20 dimension cavity is selective to exclude unwanted material. Such unwanted material will depend on the nature of the test but may include cells such as blood cells, cell debris or dispersed high-molecular-weight material. A suitable filter can be for example a paper filter located at the sample entrance to the capillary

cavity or a strip or area of filter or dialysis membrane material fixed at or in the capillary cavity. It is also within the scope of the invention that the area covered by an immobilised reagent within the capillary cavity, e.g. an immobilised antigen or antibody, can be significantly less than the whole area of the

25 capillary cavity, and arranged so that all of the sample liquid must pass over the immobilised reagent as liquid is drawn into the cavity. In this way a sample-concentration effect can be achieved, with a resulting surface concentration of relevant analyte material (e.g. complementary antibody or antigen) higher than would result from uniform adsorption over the whole capillary cell area. Such an immobilised coating over a restricted threshold area of the capillary cell surface can form in effect a selective barrier to allow passage

30 of sample liquid beyond the barrier with selective retention of an analyte material of interest.

In the use of devices made according to the invention, a drop of sample liquid can be placed on a collection surface of the test device, or else the device can be dipped into a quantity of liquid to be sampled. If ancillary reagents are needed they can be dosed separately, or they can be carried in dry

35 releasable form on a part of the device to be contacted in use by sample liquid, e.g. a surface of the capillary cavity or a surface of a filter if present.

Further variants and particular features of the test devices are described for example below.

The additional structure can for example be a further sheet material bonded to the first sheet material by suitable bonding adhesive, and spaced therefrom by a capillary space, e.g. less than about 1mm, to

40 allow uptake of sample liquid between the sheets by capillarity, preferably in a defined reproducible volume. Either or both sheets may be transparent to light with optically regular, generally smooth, surfaces. The separation of units can be achieved by scribing and breaking the sheet materials, e.g. of glass, siliceous or plastics material, and in the examples described below is carried out so as to leave an external loading surface to which sample liquid can be loaded or applied and from which it can be drawn onto the cavity of

45 the device. The external loading surface has preferably a capacity to contain or hold at least enough liquid (e.g. in the form of a drop of material spread out over the surface) to load the cavity fully.

In certain examples of devices made according to the invention, the cavity of the device can be a thin planar cavity between two opposite walls forming a cell, and preferably cemented or made into an integral unit. In some cases, for example, the device can include a bonded structure of transparent plates similar to the structure of an unfilled liquid crystal display device as obtained as an intermediate stage in manufacture

50 of liquid crystal displays.

Thus devices made according to the invention include those possessing a (for example disposable) translucent or transparent capillary cell, which can be made by the methods described herein, for carrying out specific binding assays, comprising a pair of opposite transparent plates spaced less than about 1mm apart, and sealed together to form an apertured liquid-retentive cell able to take up and retain by capillary

55 action a (preferably defined) volume of (usually aqueous) liquid, and carrying on at least one of its internal surfaces a coating of immobilised specific binding agent with a specificity appropriate to the assay to be carried out. "Defined volume" means a volume that is determined substantially by the shape and configuration of the cell itself and not appreciably by the volume of sample if applied in excess.

It is important to have an accurately defined (parallel) cavity of capillary gap dimensions in the devices of this invention, but not so important that the total volume taken up by the capillary gap should be defined: the important parameter is rather the volume dosing per unit area of the optical reagent bearing surface of the waveguide, and this is achieved by an accurately defined parallel spacing of the opposite walls of the cavity in the device. The spacing is preferably within the broad range about 0.01 to 1mm, eg. of the order of 0.1 mm, eg. 0.03-0.3 mm, as a compromise between too wide spacings leading to excessive diffusion time of materials in the sample across the gap to the reagent-bearing surface, and too narrow spacings which may collect too little sample.

Materials suitable to form the cavity are for example glass, eg. sheet soda glass about 1mm thick, silica and plastics sheet material, eg. acrylic plastics material.

- 10 Where plastics materials are used to form the capillary cells, they can for example be used in the form of precision mouldings, e.g. provided with spacers such as ridges to achieve controlled spacing of the component walls of the capillary cell cavities.

- 15 In some cases the cell has an outer surface portion or lip to which a quantity of sample sufficient to fill the cell can be applied and from which it can easily be made to run into the capillary cell by capillary action. Such a lip can easily be formed by an extension of one of the plates, outwardly beyond the cell aperture, for a distance sufficient to give a surface area large enough for convenient sample loading. It can also if desired be given a liquid-conductive shape, such as a groove or channel leading towards the capillary cell inlet. An alternative form of inlet is one formed by an aperture in one wall of the capillary cell, e.g. a hole that exposes an area of opposite wall of the cell on to which a sample can be loaded. Selective barriers such as those described elsewhere herein, such as filters and dialysis membranes, can also be located in or adjacent to such inlet apertures, and may be pre-dosed with dry releasable reagents. It is especially convenient to incorporate these features in the plastics capillary cells, and in such cases precision-moulded apertures supplied in a moulded plastics sheet can provide the perpendicular optically flat ends of the capillary cells when the resulting assembly of plural capillary cells is separated into individual cell units.

- 25 Preferably the sealing of the cell can be achieved by using a line of epoxy resin, leaving an aperture, e.g. extending the resin along two opposite sides of a rectangular capillary cell, to give a filling aperture and a further aperture left to allow the exit of air from the capillary cell as it fills up. Suitably, the resin can comprise solid particles to ensure a desired spacing for the plates as they bed down on the resin. Particles such as substantially monodisperse ballotini (fine glass particles) of diameter about 100 micron or otherwise corresponding to the chosen capillary gap, or short glass fibre lengths of for example 8 micron diameter and 50-100 micron long (e.g. made by mortar-grinding of long glass fibre and exclusion of long residual fibres by sieving), are suitable to regulate small spacings of the order of the diameter of the ballotini or the fibres. Generally, spacings in the range 5 to 500 microns can be chosen, by way of non-limitative example.
- 35 Fibres are preferred for very narrow gaps, as they are more easily obtainable in diameters less than about 50 micron than are monodisperse ballotini: ballotini are preferred for the wider gaps.

- 40 The specific binding agent can be chosen among any of those normally used for purposes of specific binding assay, especially antigens and antibodies. Suitable examples are antiglobulin antibody, or antibody specific to human apolipoprotein A₁ or B, or anti-steroid antibody, eg. specific for oestrone-3-glucuronide or pregnanediol glucuronide, or non-immunological binding agents such as concanavalin A, or cibacron blue. They can be immobilised to the glass or silica or plastics surface in any of the ways otherwise practised for such immobilisation. For example, it can be useful simply to coat and dry the binding agent and sucrose on to the carrier surface, either simultaneously or successively. Covalent and other immobilisation can be achieved where desired in any of the ways mentioned in EP specification 0 014 530 (Unilever), and references cited therein, especially in the case of plastics materials, and in any of the ways mentioned in "Immobilised Enzymes for Industrial Reactors" (ed. Messing, Academic Press, 1975; especially Filbert, chap 3), or in for example USP 3 652 761 or GB 1 530 997, for a wide variety of carrier materials including siliceous materials such as glass and silica.

- 50 The format of immunoassays, especially e.g. fluorimmunoassays, which can be carried out in these capillary cell devices corresponds to the known formats of other immunoassays of the prior art. For example, such an assay can be a competitive assay in which an antigen analyte competes for binding sites on a specific immunoabsorbent with a fluorescent competitor, e.g. a fluorescent-labelled antigen analogue. Alternatively, the analyte can be contacted with the immunoabsorbent in the absence of the fluorescent ligand and this reaction can be followed by contact between the treated immunoabsorbent and the further alternative, a solution-phase competition assay can be carried out between an analyte and a solution-phase binding partner and fluorescent-labelled competitor, (e.g. soluble antibody and competitive

fluorescent-labelled antigen), in the presence of an immobilised antiglobulin carried on the capillary cell surface to immobilise the antibody participating in the assay, e.g. after the formation of the mixture of labelled and unlabelled immune complex.

- In a further alternative, a sandwich test or antiglobulin test can be carried out in which the immunosorbent and fluorescent ligand (e.g. both antibody, or in the antiglobulin test respectively an antigen and antiglobulin) both have specific affinity for the analyte (the antigen or antibody) under test.

In a further alternative, a more general ligand-binding reaction may be used, e.g. an immobilised layer of concanavalin A, which competitively binds glucose and a fluorescently-labelled albumin or other proteins, such as Cibacrom blue, which specifically binds fluorescent and unlabelled albumin or other proteins.

- Similarly, luminescent assays may be carried out using labels which participate in chemiluminescent or bioluminescent reactions, e.g. luciferase or horseradish peroxidase.

The accompanying Figures 1-8 and associated description are intended to illustrate the invention by way of example only.

- Figure 1 shows a diagrammatic section through a disposable capillary cell device made according to one embodiment of the invention.

Figure 2 shows a diagrammatic plan of the cell device of Figure 1, and includes a line I-I to show the line of section of Figure 1.

Figure 3 shows in diagrammatic fragmentary plan an intermediate stage in the manufacture of a plurality of devices as of Figures 1-2.

- Figure 3a is a schematic fragmentary plan corresponding to a variant on the arrangement shown in Figure 3.

Figure 4 shows in schematic perspective view a specifically-reactive capillary cell device made according to an embodiment of invention.

Figures 5 and 6 show a schematic plan and section of the device of Figure 4.

- Figures 7 and 8 show a corresponding schematic plan and fragmentary section of an alternative device, a modification of the device of Figures 4-6.

Figures 1-2 show a capillary cell device of a size to be handled easily, e.g. about 3 cm x 1.5 cm. The device comprises upper transparent (e.g. plastics, glass, or silica) plate 1 and lower transparent (e.g. similar) plate 2 (about 1 mm thick) fixed together in parallel opposed and spaced relation, less than 1 mm apart, by bonding tracks 3 of suitable (e.g. epoxy) adhesive to form a capillary cell cavity 4, open at both ends, which communicates with the outside through a first discontinuity in the bonding 3 arranged to form a cell aperture at side 5 of plate 1. Another discontinuity is present at the other end of bonding 3, to leave another aperture, to allow exit of air when a sample liquid is loaded into the cell. Plate 2 is larger than plate 1 and has a portion 6 extending away from the aperture. Portion 6 of plate 2 acts as a platform or threshold or lip onto which a drop of sample liquid can be applied, so that this liquid can be made to fill the capillary cell cavity 4 by capillary flow. Cavity 4 attracts and contains a definite and adequately reproducible volume of liquid when loaded in this way.

Immobilised to the inner surface of the capillary cell is a layer 7 of material relevant to the test procedure in which the capillary cell is to be used. In the example shown in the drawings the layer 7 is a patch of material carried on plate 2. For the purpose of immunoassay, it can be for example an area of immobilised immunosorbent sensitisation, e.g. immobilised antibody, relevant to an immunoassay. There can be more than one such layer, e.g. a layer on plate 1 as well as plate 2, or a superimposition and/or side-by-side plurality of layers on either plate. For similar or other purposes, the layer 7 or other layer(s) lining the internal surface(s) of the capillary cell can include an electrically conductive layer or layers, as described in EP-A-170375 and in such a case conductive external connections can be provided by means of conductive tracks or connectors from the interior of the cell to the exterior of the cell, if desired, passing between bonding layer 3 and the surface of the plates. These can for example be made in a manner known per se and used in the conventional surface fabrication of conductive tracks as often employed in the manufacture of semiconductors and liquid crystal displays, e.g. as referenced below.

- Either plate 1 or plate 2 or both should be transparent or translucent.
- The section shown as Figure 1 presents plates 1 and 2 spaced apart because the line of section does not extend through the bonding tracks 3.

The fabrication of a plurality of cells such as that of Figures 1-2 is illustrated by Figure 3, a fragmentary plan diagram showing an intermediate stage in the manufacture of such cells. A large plate 8 of glass or other material to make plates 2 is cleaned and coated in any appropriate way (e.g. as described below) with patches of material 7 of any of the kinds described above as well as tracks of bondable adhesive 3. A second plate, not shown, is then adpressed to plate 8, optionally after forming on it bonding tracks corresponding to track 3, and optionally after forming patches or tracks of any other desired material, and

the adhesive is cured. Then the assembly is broken or cut along lines shown as dotted lines 9 in Figure 3, and corresponding lines in the upper plate (not necessarily in registration with lines 9, though). The result is to give cells like the cells shown in Figures 1-2.

Figure 3a shows in diagrammatic plan view an alternative and preferable intermediate stage in the manufacture of capillary cell devices as in for example Figures 1 and 2. The main differences between Figures 3 and 3a, apart from variation in shape and detail of pattern of the devices to be produced, are that waste edges 31 of the sheet material 8 are provided, and tracks of bondable adhesive 3a are provided in such waste edges 31. The purpose here is to achieve better or more convenient control of the spacing between the upper and lower plates: the bondable adhesive tracks 3 and 3a contain the fibres or microspheres described above to regulate spacing of upper and lower plates and the position of tracks 3a helps to avoid irregularity of spacing at the plate edges.

The arrangements of Figures 3 and 3a have been described in relation to the use of sheet glass as a substrate material: especially but not exclusively where plastics sheet is used, it can be convenient to use other than plain sheet material, e.g. the spacer ridges, inlet apertures and filter arrangements described elsewhere herein can be incorporated as part of such sheets before the capillary cells are assembled.

The capillary cell device of Figures 1-2, among other devices made according to this invention, can if desired be provided with any convenient form of handling-piece or holder and for this purpose may be provided with any convenient form of fixed or releasable connexion arrangement to engage with such a holder where this is not formed in one piece with the cell device.

For convenience of optical measurement, it is desirable that the edge or edges of plate 1 and/or 2 which is/are optically smooth is/are perpendicular to the plane of the plate. The remaining edges may if desired be coated with black paint or other light-absorbing material. Alternatively or additionally, absorbing pigment such as carbon black may be incorporated in the cement such as epoxy that is used to bond the two plates together.

In use, the device of Figures 1 and 2 can transmit fluorescence from an adsorbed fluorescent material along either or both of the plates 1 and 2 in the manner of a waveguide or optical fibre. Transfer of useful light across the boundary of the plate can occur by the evanescent wave located very close to the interface. If desired, a thin layer of for example silica or magnesium fluoride, of the order of a quarter-wavelength thick at the wavelength of interest, may be fabricated on to the plate before deposition of the biochemical reagent, in order to improve light transfer across the boundary. The thickness and refractive index of the dielectric layer are preferably together chosen to maximise the intensity of the evanescent wave associated with light transmitted in the plate in a path that corresponds to total internal reflection near (and slightly above) the critical angle for the boundary between the plate material and the sample liquid, e.g. usually in the range about 1° - 5° above the critical angle. The optimum refractive index of the dielectric layer is as close as possible to that of the sample liquid: practically it is most usual to choose a dielectric of magnesium fluoride ($n = 1.38$) or silica ($n = 1.46$) when glass is used. The optimum thickness for the layer is expressed in terms of the angle of incidence P of the light in the plate at the plate-dielectric interface, and the refractive indices of the media: n_1 of the sample liquid, n_2 of the plate, n_3 of the dielectric, L is the wavelength of the light in use:-

$$t = L/4 \cdot \frac{\left[1 - \frac{2}{\pi} \arccos \sqrt{\frac{n_3^2 - n_2^2 \sin^2 P}{n_3^2 - n_1^2}} \right]}{\sqrt{(n_3^2 - n_2^2 \sin^2 P)}}$$

In further derived alternative examples, continuous or discontinuous thin metal layers e.g. of silver or indium, preferably overlain by a vacuum-deposited very thin corrosion-resisting layer such as silica, may also be deposited on either or both plates to enable the device to be used in corresponding other methods of optical analysis known in themselves, which exploit such layers, e.g. the per se known phenomenon of surface plasmon resonance, as described in B.Liedberg et al., *Sensors and Actuators*, 4 (1983) 299-304. In any of these cases the immobilised layers of biochemical reagents may be supplemented or replaced by releasable reagent coatings, e.g. formed by air-drying protein-sucrose mixtures in thin films on the plates: selected and combined according to the particular test chemistry to be performed in the device. The range of chemical or binding reactions that can form part of the tests to be carried out spans the range of known binding-reaction tests, and includes enzyme-linked, fluorescence, luminescence, binding and quenching

reactions of any kind based on solid-phase immunoadsorbent or other specific binding adsorbent.
Further details of procedures and material to fabricate cell devices such as the capillary cell device of
Figures 1-2 are given in the following Example, further illustrating embodiments of this invention.

5

Example

A sheet of (e.g. soda) glass for example about 1mm thick, and large enough to contain a 2-dimensional array of cell areas, with a plurality of several cell units in each direction, is cleaned by any suitable method, eg. by detergent and ultrasonic treatment and it need be by solvent vapour degreasing in known manner, or
10 by successive hot (80°C) treatments with ammonia hydrogen peroxide and hydrochloric acid/hydrogen peroxide, water-rinsing and airdrying, e.g. at 115°C for 30 minutes. A pattern of patches of a desired protein or other coating is then applied by the following or equivalent technique. Covalent coupling of antigen or antibody or other protein is achieved by first reacting the glass with a silane-based coupling
15 compound in known manner. A suitable such reagent is for example, as used here, a terminal amino-alkyl trimethoxysilane, e.g. the 3-aminopropyl compound, used at a concentration of suitably about 2% v/v in acetone. In alternative methods, another reagent substantially as described in USP 3 652 761 can be used instead. After reaction with the amino-silane reagent, the amino terminals immobilised on to the glass are in turn reacted with (e.g. 2% pH 7) glutaraldehyde, excess reagents are removed and the activated glass with
20 immobilised aldehyde groups is reacted with the protein in solution (e.g. 1 mg/ml antibody immunoglobulin), according to component techniques well known in themselves. Other proteins can be applied at choice in concentrations of the order of 0.1-1 mg/ml. Treatment at about pH 9.5 for 2 hours at 37°C has been found suitable here. A suitable final active protein loading rate on the glass surface can be for example about 0.5 microgram/cm². This is thought to constitute a continuous or near-continuous layer.
25 The dosage or density or specific activity of the immobilised layer is determined by the sensitivity requirements of the particular assay chemistry, which in itself forms no part of this invention. Excess reagents can be removed for example washing in strong buffer (0.1M acetate, 0.5M NaCl, pH4-5), then neutral buffer washing, (pH 7-7.4), followed by pH 9-10 washing and neutralisation, e.g. with neutral tris buffer.

30 An alternative and sometimes preferred method for coupling protein to glass employs an epoxy-silane reagent, especially the glycidyoxypropyltrimethoxysilane (e.g. 2% v/v in toluene, 70°C for 23 hours), according to DP Herman et al., J.Chromatogr.Sci., (1981), 19 (9) 470-6. With this reagent the use of aldehyde reagents can be omitted, as the epoxysilylated glass can react directly with protein.

It is usually desirable to apply a stabilising coating to such layer-coated surfaces such as a coating of a solid humectant such as sucrose. A suitable example of such a coating is an 8 micron-thick solid sucrose
35 coating applied for example by spin-coating the plate with sucrose solution and drying in air.

Releasable coatings can be applied with per se known composition, e.g. carried in admixture with a solid humectant, such as sucrose glaze. We find that it can be desirable to include detergent or inert protein in such coatings (or a soluble salt or buffer material) especially where the active material to be released is
40 itself a protein, and it can be especially desirable to avoid large excess of the reagents in such releasable coatings in relation to the test reactions in which they are to take part.

Uniformity of the coating processes can be important, and if appropriate can be checked by test procedures in which radiolabelled and/or fluorescent protein is coated, and then optionally reacted with a further and preferably differently-labelled binding agent. Uniformity of the coating and of its binding capacity
45 can then be checked by surface fluorescent measurement and/or surface radioactivity measurement, eg. using a laboratory gamma-ray scanner.

If it is afterwards desired to etch the coating on the glass, the coated glass is then placed in a confined atmosphere substantially free of moisture or air draughts, e.g. it can be brought close to another flat inert surface to reduce the air gap on the coated side to about 1mm or less. The glass is then illuminated with an
50 ultraviolet patterned image (using preferably light of as narrow as practicable a waveband around c. 280nm) in a pattern corresponding to areas from which the coating is to be etched away, e.g. a grid pattern, to leave a pattern of surviving protein patches. Illumination can for example be carried out using a GE 7-watt mercury lamp spaced a few centimetres from the plate, for a period of about 5-20 minutes. The illumination pattern can be produced by masking close to the plate, or by a real imaging system. The ultraviolet etching
55 used here relies on the same principle as the u.v. etching process described by J A Panitz, I Glavier, in Surface Science, 97 (1980) pp 25-42, to which reference is made.

Then a uv-curable epoxy adhesive is printed on to the coated, eg. patch-coated, glass plate in a desired pattern for forming a connexion with an upper spaced plate. The epoxy adhesive is applied by a silk-screen

technique which is conventional in itself, and in itself forms no part of this invention.

The epoxy resin can have a small content of short-length glass fibre, about 20 micron in diameter and about 100-200 micron long, (made for example by grinding long glass fibre in a mortar and sieving to remove residual long fibres). A preferred alternative to the glass-fibre pieces is a content of ballotini in the epoxy resin, used as follows. In order to produce a gap of for example 100 micron, correspondingly-sized ballotini are incorporated in the epoxy; a layer of epoxy a little thicker than the desired spacing between the plates, e.g. 10% thicker, e.g. about 110 micron for a desired spacing of 100 micron, can be laid down by screen-printing, and the addition plate pressed gently into position to spread the epoxy slightly.

If desired, a mirror-image of the first pattern of epoxy adhesive can be applied as a pattern to a second similar sheet of glass, either coated patchwise with the same or a different protein or other coating material, or otherwise uncoated, and the two sheets then brought together, subjected to vacuum or deoxygenation if needed for curing, and cured by ultraviolet illumination. The ultraviolet is applied as an image with a pattern that avoids the patches of coated protein or other material which are to be retained in active form.

After adhesive curing, the two glass plates can be scribed and broken down into individual cell units in any convenient known manner as used in stages in the manufacture of liquid crystal devices, and particular by the methods referred to in Specifications Nos. CH-627 559, and 829 002, concerning fabrication of liquid crystal display devices. Corresponding steps in the methods of those specifications and of this invention can be performed by similar methods, mutatis mutandis.

A convenient form of cell obtainable by this process comprises two substantially parallel opposed layers of glass, air-spaced by about 5-500 micron, which, together with an incomplete frame of bonding material located between them, (having at least one opening for the inward passage of liquid and possibly also the outward passage of air), form a capillary cell able to take up a defined volume of aqueous liquid. Preferably one of the glass layers extends out beyond the opening of the cell to enable a drop of liquid to be placed on its surface and pass either wholly or partly into the cell.

Figure 4 shows in schematic perspective a further example of a specifically-reactive capillary cell device made according to an embodiment of the invention. The device shown is a disposable single-use test device for carrying out microchemical testing on very small liquid samples. Figure 5 (not to scale) shows a corresponding plan view and Figure 6 a schematic section along lines 6'-6" in Figure 5. The device of Figures 4-6 comprises an upper glass plate 1 and a lower glass plate 2 related as plates 1 and 2 of Figure 1. A reactive layer as 7 in Figure 1 is present on the surface of plate 1, so that the reagent dissolves in a releasable coating on the opposite wall of the cell, i.e. the surface of plate 2, so that the reagent dissolves in the sample liquid drawn into the cell. The device of Figures 4-6 can be handled by the user by a handlepiece 42 of a supporting and handling frame indicated generally 41 and made of any convenient plastics moulded material. Arms 43 serve to support the cell assembly and locate it in relation to the operative parts of an optical instrument for optical analysis of the contents of the sample cell. End 44 of plate 2 is optically clear, flat and perpendicular to the plane of plate 2, to allow exit of light arising from the contents of the capillary cell between plates 1 and 2. The other edges of plates 1 and 2 are in this embodiment usefully painted black, and epoxy bonding tracks 3, corresponding to tracks 3 in Figure 1, besides their content of 100 micron ballotini to space plates 1 and 2 apart, also contain carbon black granules to help minimise stray light. A filter paper rectangle 45 is located on a part 46 of plate 2 that extends beyond the length of plate 1 to form a sample-receiving inlet: the adjacent open end 47 of the capillary gap between plates 1 and 2 is an inlet for the capillary cell delimited by plates 1 and 2 and bonding tracks 3, and filter 45, of a grade sufficiently fine to exclude the passage of red blood cells, is in contact with open inlet end 47 and preferably slightly underlaps plate 2 at end 47. Filter 45 is retained in place if desired by adhesive along parallel lines continuing tracks 3, and can if desired be impregnated with a releasable/auxiliary reagent such as for example a pH buffer salt. Portion 47 of plate 2 extending beyond plate 1 to the optical end 44 of plate 2 can if desired be given a hydrophobic coating.

In use, a sample of liquid to be tested, e.g. a drop of whole blood, can be applied to the inlet zone formed by filter 45, and from such a drop a quantity of relatively cell-free liquid is drawn into the capillary cell. Here take place the binding or other e.g. enzyme reactions appropriate to the nature of the required test and corresponding reagents previously carried in prepared dry form on one or both of the opposite internal cell walls formed by plates 1 and 2, and the loaded cell can then for example be put into a photometric instrument, e.g. that described in EP-A-170376 for optical measurement as described therein.

Figures 7 and 8 illustrate a modification of the device of Figures 4-6. The device has two snap-on handle-and-support pieces, one shown at 41, corresponding to 41 in Figures 4-6 except that it is detachable and another handle and support piece 71 comprising handle 72, which is a removable snap-on fit over lateral ribs 73 at the optical end of the device, which comprises similar optical end-faces 44 and 74 of both

the spaced-apart plates 1 and 2. Handle 42 is a firmer snap-on fit than handle 72.

the spaced-apart plates 1 and 2. Handle 42 is a firmer snap-on fit than handle 72. In this condition a cellulose nitrate or acetate microfine filter or dialysis membrane 75 is exposed at the inlet end of a capillary cell defined as before between plates 1 and 2 and binding tracks 3, but defined here also by a further transverse blackened epoxy bonding track 3a that limits the forward spread of liquid towards ends 44 and 74 of plates 1 and 2, and results in the forward parts of plates 1 and 2 being able to constitute waveguide portions bounded by air on each side. Two lateral gaps are left between tracks 3 and 3a and two corresponding apertures 78 in frame 73 allow for exit of air when the capillary cell fills with sample liquid. Thus, in use, the device can be held by handle 72 and dipped into a source of sample liquid to allow a sample to fill the capillary cell through filter 75. In this embodiment each of plates 1 and 2 carries an immobilised reactive layer 7 and 77 such as an appropriate antibody layer fabricated as described above. If desired, the extent of reactive layers 7 and 77 can be limited to the area shown hatched in Figure 7 on each of plates 1 and 2 between filter 75 and line 78. With such an arrangement, the gently inflowing sample liquid passes over the reactive layers which are each able to abstract their respective ligand, the surface concentration of which can therefore be concentrated in the hatched region.

[illegible]

20 device. A corresponding optical measuring instrument described in EP-A-170376, which is to be measured optically by the photometric arrangements and methods described in EP-A-170376.

Claims

- Claims
- 30 1. A process of manufacturing specifically-reactive sample-collecting and testing devices, comprising the steps of (a) forming an immobilised specifically-reactive coating on the surface of a transparent sheet material which is capable of acting as a light transmissive waveguide and which is to provide a part of a multiplicity of the devices, (b) attaching to the said sheet material an additional structure which together with said coated sheet material provides for each device of the multiplicity of devices a cavity of capillary dimension for collecting and retaining by capillarity a volume of sample liquid in contact with the specifically-reactive coating and (c) separating the assembled laminate into portions each providing one or a plurality of the sample-collecting and testing devices, such that the transparent sheet material of each device has at least one edge transverse to the plane of the sheet which is substantially optically smooth.
 - 40 2. A process according to claim 1, in which the specifically-reactive coating is provided as a pattern of discrete patches.
 3. A process according to claim 2, in which patches forming the pattern are made by first forming a continuous coating and then removing portions of it to leave the desired pattern of the discrete patches.
 - 45 4. A process according to any of claims 1 to 3, wherein the specifically-reactive coating is selected from an antigen, an antibody or a derivative thereof.
 - 50 5. A process according to any of claims 1 to 4, wherein one or more ancillary reagents are applied to the sheet material or to the additional structure such that in each device obtained a part of the device to be contacted in use by sample liquid carries the ancillary reagent(s) in dry releasable form.
 - 55 6. A process according to any of claims 1 to 4, wherein fluorescent ancillary reagent is applied to the sheet material or to the additional structure.
 7. A process according to any of claims 1 to 6, wherein prior to application of the immobilised coating, a thin coating of conductive material capable of showing a surface plasmon resonance effect is applied to

the surface of the said sheet material which is to carry the said Immobilised coating.

8. A process according to claim 7, wherein the thin coating of conductive material is a coating of silver up to about 50 nm thick.
9. A process according to any of claims 1 to 6, wherein prior to application of the immobilised specifically-reactive coating a thin dielectric layer is deposited on the surface of the transparent sheet material which is to carry the said immobilised coating.
10. A process according to claim 9, wherein the thin dielectric layer comprises magnesium fluoride or silica.
11. A process according to any of claims 1 to 10, wherein the additional structure is a further sheet material and is bonded to the first sheet material by a suitable bonding adhesive, and spaced therefrom by a capillary space to allow uptake of sample liquid between the sheets by capillarity.
12. A process according to any of claims 1 to 11, wherein the device is provided with an external surface to which sample liquid can be loaded or applied and from which it can be drawn into the cavity.
13. A process according to any one of claims 1 to 12, comprising the further step of providing each device with an electrode structure for making electrical measurements, in use, on the contents of the capillary cell.

Revendications

1. Un procédé de fabrication de dispositifs spécifiquement réactifs pour la collection et l'analyse d'échantillons, caractérisé en ce qu'il comprend les étapes de (a) formation d'un revêtement spécifiquement réactif immobilisé sur la surface d'un matériau en feuille transparent qui est capable de jouer le rôle d'un guide d'ondes transmettant la lumière et qui doit fournir une partie d'une multiplicité de dispositifs, (b) fixation à ce matériau en feuille d'une structure supplémentaire qui, avec ce matériau en feuille enduit, fournit pour chaque dispositif de la multiplicité de dispositifs, une cavité de dimension capillaire pour collecter et retenir par capillarité un volume de liquide échantillon en contact avec le revêtement spécifiquement réactif et (c) séparation du feuilleté assemblé en parties fournissant le matériau transparent en feuille de chaque dispositif présente au moins un bord transversal par rapport au plan de la feuille qui est pratiquement optiquement lisse.
2. Un procédé suivant la revendication 1, caractérisé en ce que le revêtement spécifiquement réactif est fourni sous forme d'un motif de patches discontinus.
3. Un procédé suivant la revendication 2, caractérisé en ce qu'on fait des patches formant le motif en formant d'abord un revêtement continu, puis en éliminant des parties de celui-ci pour laisser le motif désiré de patches discontinus.
4. Un procédé suivant l'une quelconque des revendications 1 à 3, caractérisé en ce que le revêtement spécifiquement réactif est choisi parmi un antigène, un anticorps ou un dérivé de ceux-ci.
5. Un procédé suivant l'une quelconque des revendications 1 à 4, caractérisé en ce qu'un ou plusieurs réactifs auxiliaires sont appliqués sur le matériau en feuille ou sur la structure supplémentaire de telle sorte que dans chaque dispositif obtenu, une partie du dispositif à mettre en contact avec le liquide échantillon en cours d'utilisation, porte le ou les réactifs auxiliaires sous une forme sèche pouvant être libérée.
6. Un procédé suivant l'une quelconque des revendications 1 à 4, caractérisé en ce qu'un réactif auxiliaire fluorescent est appliqué sur le matériau en feuille ou sur la structure supplémentaire.
7. Un procédé suivant l'une quelconque des revendications 1 à 6, caractérisé en ce qu'avant application

du revêtement immobilisé, un enduit mince de matière conductrice capable de présenter un effet de résonance de plasmon de surface est appliqué sur la surface de ce matériau en feuille qui doit porter ce revêtement immobilisé.

- 5 8. Un procédé suivant la revendication 7, caractérisé en ce que l'enduit mince de matière conductrice est un enduit d'argent ayant jusqu'à 50 nm d'épaisseur.
9. Un procédé suivant l'une quelconque des revendications 1 à 6, caractérisé en ce qu'avant application du revêtement immobilisé spécifiquement réactif, une mince couche diélectrique est déposée sur la surface de ce matériau transparent en feuille qui doit porter ce revêtement immobilisé.
- 10 10. Un procédé suivant la revendication 9, caractérisé en ce que la mince couche diélectrique comprend du fluorure de magnésium ou de la silice.
- 11 11. Un procédé suivant l'une quelconque des revendications 1 à 10, caractérisé en ce que la structure supplémentaire est un autre matériau en feuille et est liée au premier matériau en feuille par un adhésif de liaison convenable, et séparée de celui-ci par un espace capillaire pour permettre la prise de liquide échantillon entre les feuilles par capillarité.
- 12 12. Un procédé suivant l'une quelconque des revendications 1 à 11, caractérisé en ce que le dispositif est pourvu d'une surface externe sur laquelle le liquide échantillon peut être chargé ou appliqué et à partir de laquelle il peut aller dans la cavité.
- 13 13. Un procédé suivant l'une quelconque des revendications 1 à 12, caractérisé en ce qu'il comprend l'étape supplémentaire selon laquelle chaque dispositif est pourvu d'une structure d'électrode pour faire des mesures électriques, en cours d'utilisation, sur le contenu de la cellule capillaire.

Ansprüche

- 30 1. Herstellungsverfahren für spezifisch-reaktive Probensammelungs- und Testvorrichtungen, umfassend die Schritte von
 - (a) Bildung einer immobilisierten spezifisch-reaktiven Beschichtung auf der Oberfläche eines transparenten Plattenmaterials, das geeignet ist, sich als lichtdurchlässige Wellenführung zu verhalten und das für einen Teil der Vielfalt der Vorrichtungen vorzusehen ist,
 - 35 (b) Anbringen eines zusätzlichen Aufbaues an das Plattenmaterial, der zusammen mit dem beschichteten Plattenmaterial für jede Vorrichtung der Vielfalt von Vorrichtungen einen Hohlraum von kapillarem Ausmaß vorsieht, der durch die Kapillarität ein Volumen von Probenflüssigkeit in Kontakt mit der spezifisch-reaktiven Beschichtung sammelt und festhält und
 - 40 (c) Trennen der zusammengesetzten Schichtung in Teile, wobei Jedes mit einem oder einer Vielzahl von Probensammelungen und Testvorrichtungen versehen ist, so daß das transparente Plattenmaterial jeder Vorrichtung mindestens eine Kante hat, die quer zur Fläche der Platte verläuft, die im wesentlichen optisch glatt ist.
- 45 2. Verfahren nach Anspruch 1, in dem die spezifisch-reaktive Beschichtung als ein Muster von einzelnen Flecken vorgesehen ist.
3. Verfahren nach Anspruch 2: in dem die Flecken, die das Muster bilden, zuerst durch das Bilden einer durchgehenden Beschichtung in dem die Flecken, und dann durch das Entfernen von Teilen davon, um das gewünschte Muster der einzelnen Flecken überzulassen, erstellt werden.
- 50 4. Verfahren nach einem der Ansprüche 1 bis 3, wobei die spezifisch-reaktive Beschichtung aus einem Antigen, einem Antikörper oder einem Derivat davon ausgewählt wird.
- 55 5. Verfahren nach einem der Ansprüche 1 bis 4, wobei ein oder mehrere Zusatzreagentien an das Plattenmaterial oder den zusätzlichen Aufbau

aufgetragen werden, so daß in jeder Vorrichtung ein Teil der Vorrichtung erhalten wird, der bei Verwendung einer Probenflüssigkeit, die das Zusatzreagens bzw. die Zusatzreagentien in trockener, freisetzbarer Form trägt, in Kontakt gebracht wird.

- 5 6. Verfahren nach einem der Ansprüche 1 bis 4,
wobei ein fluoreszierendes zusätzliches Reagens auf das plattenmaterial oder den zusätzlichen Aufbau
aufgebracht wird.
7. verfahren nach einem der Ansprüche 1 bis 6,
wobei vor dem Aufbringen der immobilisierten Beschichtung ein dünner Überzug von leitendem
10 Material, das geeignet ist, einen Oberflächenplasmonenresonanzeffekt zu zeigen, auf die Oberfläche
des Plattenmaterials aufgetragen wird, das die immobilisierte Beschichtung tragen soll.
8. Verfahren nach Anspruch 7,
15 wobei der dünne Überzug des leitenden Materials eine bis zu etwa 50 nm dicke Silberbeschichtung ist.
9. Verfahren nach einem der Ansprüche 1 bis 6,
wobei vor dem Aufbringen der immobilisierten, spezifisch reaktiven Beschichtung eine dünne dielektri-
sche Schicht auf der Oberfläche des transparenten Plattenmaterials abgesetzt wird, die die immobili-
20 sierte Beschichtung tragen soll.
10. Verfahren nach Anspruch 9,
wobei die dünne dielektrische Schicht Magnesiumfluorid oder Siliciumdioxid umfaßt.
- 25 11. Verfahren nach einem der Ansprüche 1 bis 10,
wobei der zusätzliche Aufbau ein weiteres Plattenmaterial ist und an das erste Plattenmaterial durch
einen geeigneten Haftkleber angeklebt ist, und davon durch einen Kapillaren Raum beabstandet ist, um
durch die Kapillarität zwischen den Platten die Aufnahme von Probenflüssigkeit zu ermöglichen.
- 30 12. Verfahren nach einem der Ansprüche 1 bis 11,
wobei die Vorrichtung mit einer äußeren Oberfläche versehen ist, auf die die Probenflüssigkeit
aufgeladen oder aufgebracht werden kann und von der sie in den Hohlraum gesogen werden kann.
- 35 13. Verfahren nach einem der Ansprüche 1 bis 12, umfassend den weiteren Schritt, jede Vorrichtung mit
einem Elektrodenaufbau zu versehen, um elektrische Messungen, während des Gebrauchs, am Inhalt
der Kapillaren Zellen durchzuführen.

Fig. 1.

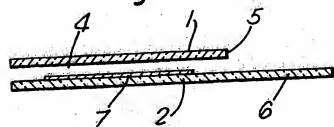


Fig. 2.

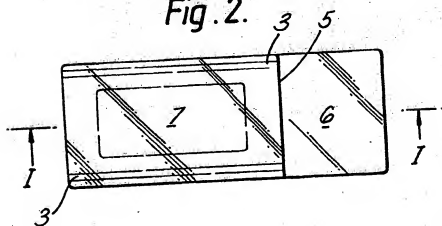


Fig. 3.

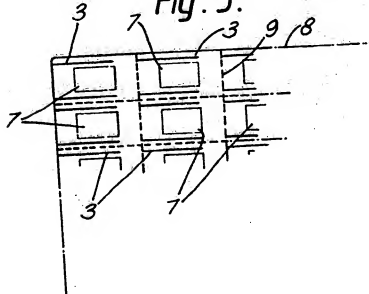


Fig. 3a.

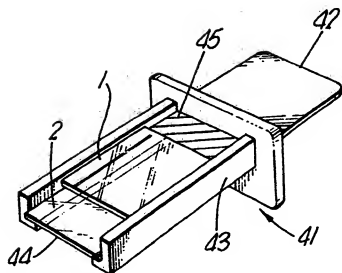
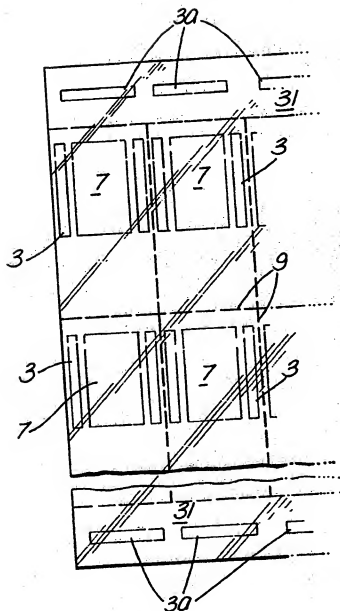


Fig. 4.

